

Fine-scale population genetic structure of a wildlife disease vector: the southern house mosquito on the island of Hawaii

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Abstract

The southern house mosquito, *Culex quinquefasciatus*, is a widespread tropical and subtropical disease vector. In the Hawaiian Islands, where it was introduced accidentally almost two centuries ago, it is considered the primary vector of avian malaria and pox. Avian malaria in particular has contributed to the extinction and endangerment of Hawaii's native avifauna, and has altered the altitudinal distribution of native bird populations. We examined the population genetic structure of *Cx. quinquefasciatus* on the island of Hawaii at a smaller spatial scale than has previously been attempted, with particular emphasis on the effects of elevation on population genetic structure. We found significant genetic differentiation among populations and patterns of isolation by distance within the island. Elevation per se did not have a limiting effect on gene flow; however, there was significantly lower genetic diversity among populations at mid elevations compared to those at low elevations. A recent sample taken from just above the predicted upper altitudinal distribution of *Cx. quinquefasciatus* on the island of Hawaii was confirmed as being a temporary summer population and appeared to consist of individuals from more than one source population. Our results indicate effects of elevation gradients on genetic structure that are consistent with known effects of elevation on population dynamics of this disease vector.

Keywords: avian malaria, *Culex quinquefasciatus*, elevation, gene flow, Hawaii, isolation by distance, microsatellite, mosquito, population structure

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Introduction

Environmental conditions, including important climatic variables of temperature and precipitation, change sharply with altitude; thus, elevation plays a significant role in determining population and community structure in a variety of organisms (Whittaker 1975). Terrestrial insects, in particular, should be strongly affected by elevation gradients because of the dependence of important life history variables, such as growth rate and development time, on temperature (Hodkinson 2005). Despite significant interest in the effects of elevation on insect populations, including

the development of adaptive clines, surprisingly few studies have explicitly addressed its role in influencing population genetic structure in insects (Levitan 1978; Etges 1984; Burla *et al.* 1986; Liebherr 1986; Peterson 1995). Here, we use microsatellite DNA variation to investigate the fine-scale population structure of the southern house mosquito, *Culex quinquefasciatus* Say, on the island of Hawaii, where this mosquito acts as a primary vector of avian disease (Atkinson *et al.* 2005; LaPointe *et al.* 2005). In particular, we focus on elucidating how populations are structured along elevation gradients. Although altitude is known to affect mosquito population dynamics and disease epidemiology in this system (Warner 1968; van Riper *et al.* 1986; LaPointe 2000; Ahumada *et al.* 2004), its effects on vector population genetic structure have not yet been explored. Furthermore, elucidating the fine-scale population structure of this mosquito is a necessary component of understanding the avian disease system in Hawaii. Vector

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population structure and movement patterns have clear implications for the ecology and evolution of vector-borne disease systems, as well as for disease epidemiology and control (May & Anderson 1979; Gauthier & Tibayrenc 2005). Because many vectors are small and vagile it is often difficult to characterize population structure and movement directly in the field, either by observation or mark-recapture (Reisen *et al.* 2003). However, geographical patterns of genetic variation in the vector can be used to draw robust inferences about these important variables (Tabachnick & Black 1996).

Culex quinquefasciatus is a widespread mosquito that is primarily domestic and can transmit a number of human and animal diseases including periodic lymphatic filariasis, West Nile virus, dog heartworm, avian malaria (Nasci & Miller 1996), and likely avian pox (Akey *et al.* 1981; Atkinson *et al.* 2005). Prior to European contact, no mosquito species were present in the Hawaiian Islands (Warner 1968). Historical records indicate that *Cx. quinquefasciatus* was introduced to the Islands in the early 19th century (Dine 1904) although the first recorded cases of avian pox and malaria were not observed until the mid-20th century (Warner 1968). Since the establishment of the disease transmission cycle, avian malaria has contributed substantially to the endangerment and extinction of native Hawaiian birds (Warner 1968; van Riper *et al.* 1986; Atkinson *et al.* 1995).

Mosquitoes in the Hawaiian Islands, particularly on the island of Hawaii itself, are distributed along steep elevational gradients (Warner 1968; Goff & van Riper 1980; LaPointe 2000). Elevation in turn plays an important role in determining vector population densities as well as the spatial dynamics of avian malaria (Warner 1968; van Riper *et al.* 1986; LaPointe 2000; Ahumada *et al.* 2004). Decreasing temperatures at higher elevations increase the development time of *Cx. quinquefasciatus* (Rueda *et al.* 1990) and place an upper altitudinal limit on the distribution of local populations (Ahumada *et al.* 2004). Furthermore, the abundance and spatial distribution of larval habitats change with altitude, becoming less common and more patchily distributed with increasing elevation (Goff & van Riper 1980; van Riper *et al.* 1986). In turn, disease has effectively forced most native bird species to seek refuge at high elevations where mosquito populations are sparse or temporary (van Riper *et al.* 1986), and where development of the malaria parasite in the vector is also limited by low temperatures (Patz & Reisen 2001; Benning *et al.* 2002).

Here, we examine how elevational gradients on the island of Hawaii act to structure mosquito populations across relatively small geographical distances. Specifically, we examine the hypotheses that (i) elevation acts as a barrier to gene flow among populations of *Cx. quinquefasciatus*, (ii) genetic structure and genetic diversity of populations differ between low and mid elevations, and (iii) bottlenecks occur in mid-elevation, forest-dwelling populations,

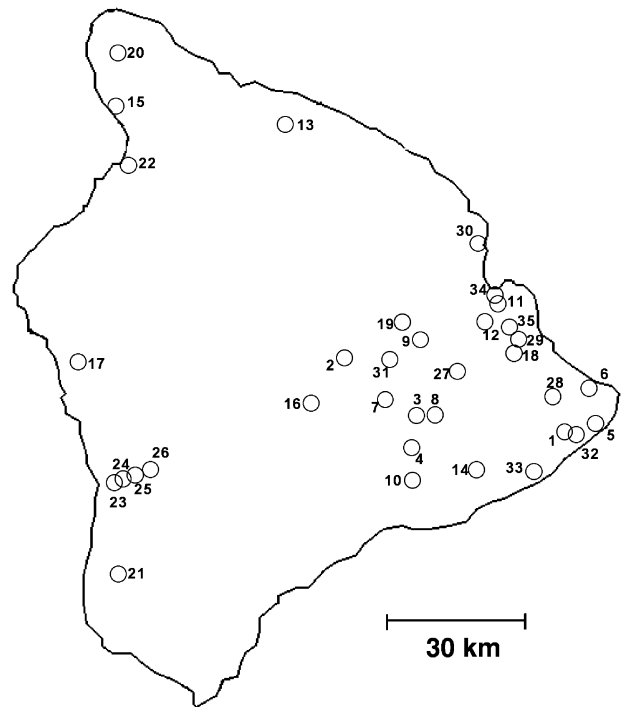


Fig. 1 Locations of sample sites on the island of Hawaii.

which may be subject to local extinction–recolonization events. We also test hypotheses regarding (iv) the origin of a group of mosquitoes recently sampled at high elevation (> 1475 m), above the predicted upper altitudinal range of the species.

Methods

Sample collection

Our study includes samples of *Culex quinquefasciatus* collected from 35 different locales on Hawaii, with many of the samples taken from the eastern side of the island along an elevation gradient on Mauna Loa and Kilauea volcanoes (Fig. 1; Table 1). The sampled populations can be divided into three regions of the island (East, North, and West, Table 1). Some samples, collected in 1997 and 1998, are from an earlier study (Fonseca *et al.* 2000), as indicated in Table 1, and detailed information on their collection and processing was previously reported (Fonseca *et al.* 2000). Some samples were collected by vector control personnel of the Hawaii District Health Office (State of Hawaii, Department of Health), in 1998 and 1999 (Table 1). These mosquitoes were collected as adults using New Jersey light traps and stored in 95% ethanol until processing. Finally, some of our samples were collected as part of an ongoing collaborative study of the dynamics of avian malaria in Hawaii (Biocomplexity of Introduced Avian Diseases in

Table 1 Specimen collections and genetic diversity. Collection refers to the source of the collected specimens: Fonseca indicates the study of Fonseca *et al.* (2000), HDHO indicates Hawaii District Health Office, and B-C indicates Biocomplexity of Introduced Avian Diseases in Hawaii project. Elevation classes are: Low = 0–305 m, Mid = 900–1350 m, High \geq 1475 m. *indicates sites at elevations of 425–760 m, which were not included in comparisons of genetic structure among elevation classes. *N* is the number of individuals typed. Average H_E is the average expected heterozygosity averaged over loci. Allelic richness is calculated according to Petit *et al.* (1998) and is averaged over loci. qGT14 null indicates the estimated frequency of the null allele at locus qGT14

Sample site	No. on Fig. 1	Collection	Region	Elevation class	<i>N</i>	Average H_E	Allelic richness	qGT14 null
Ainahou	10	Fonseca	East	Mid	10	0.655	3.55	0.19
Bryson's	1	B-C	East	Low	46	0.630	3.59	0.13
CJ Ralph 2003	2	B-C	East	High	8	0.588	3.36	0.27
CJ Ralph 2004	2	B-C	East	High	46	0.643	3.65	0.26
Cooper's	3	B-C	East	Mid	46	0.585	3.42	0.14
Crater Rim	4	B-C	East	Mid	37	0.572	3.31	0.13
Hilo Airport	11	HDHO	East	Low	46	0.608	3.51	0.14
Hilo Golf	12	HDHO	East	Low	22	0.618	3.56	0.37
Hilo Zoo	35	Fonseca	East	Low	20	0.619	3.61	0.10
Honokaa	13	HDHO	North	Low	28	0.636	3.64	0.22
Kalapana	14	Fonseca	East	*	7	0.535	3.16	0.07
Kawaihae	15	HDHO	North	Low	9	0.600	3.32	0.32
Keaau	18	HDHO	East	Low	45	0.621	3.58	0.10
Keauhou†	16	Fonseca	East	High	6	0.609	3.53	0.12
Kealakekua	17	HDHO	West	*	20	0.559	2.81	0.00
Kipuka Culex	19	Fonseca	East	Mid	22	0.596	3.29	0.33
Kohala	20	Fonseca	North	Mid	36	0.585	3.26	0.09
MalamaKi	5	B-C	East	Low	45	0.606	3.44	0.23
Manuka	21	Fonseca	West	*	6	0.614	3.35	0.33
MaunaLani	22	HDHO	North	Low	24	0.642	3.59	0.20
McCandless1	23	Fonseca	West	*	20	0.585	3.36	0.36
McCandless2	24	Fonseca	West	Mid	34	0.590	3.35	0.15
McCandless3	25	Fonseca	West	Mid	20	0.612	3.48	0.27
McCandless4	26	Fonseca	West	Mid	28	0.571	3.30	0.25
Mt. View	27	HDHO	East	*	30	0.589	3.41	0.26
Nanawale	6	B-C	East	Low	46	0.598	3.47	0.09
Pahoa	28	Fonseca	East	Low	10	0.621	3.21	0.00
Panaewa	29	HDHO	East	Low	46	0.631	3.63	0.14
Pepeekeo	30	HDHO	East	Low	39	0.611	3.49	0.24
Puu Makaala	31	Fonseca	East	Mid	24	0.584	3.30	0.17
Puu Unit	7	B-C	East	Mid	46	0.595	3.48	0.14
RT.137 A	33	Fonseca	East	Low	7	0.621	3.28	0.23
RT.137B	32	Fonseca	East	Low	8	0.683	3.71	0.18
Village	8	B-C	East	Mid	49	0.594	3.45	0.18
Waiakea	9	B-C	East	Mid	46	0.575	3.39	0.14
Waiakea Hlth Ctr.	34	HDHO	East	Low	11	0.621	3.72	0.10

†this site was misspelled as Keahou in Fonseca *et al.* (2000).

Hawaii; Woodworth *et al.* 2005) in 2002–04 (Table 1). In these sites, modified miniature Centers for Disease Control and Prevention (CDC) light traps baited with dry ice (CO₂) and gravid traps baited with an alfalfa infusion were used to capture adult mosquitoes. The heads and abdomens of these mosquitoes were removed for pathogen diagnostics and the thoraxes were stored individually in 95% ethanol for subsequent DNA extraction for use in this study. Dissection tools were cleaned with 50% bleach solution, rinsed, and flamed dry to prevent contamination between

specimens. Each 'Biocomplexity' sampling locale consisted of a 1-km square study grid containing 25 trapping stations that were spaced at least 100 m apart and whose location was determined using systematic-random assignment. Samples from different stations within a given 1-km grid were pooled in our analyses.

One of the 'Biocomplexity' study sites (CJ Ralph) occurs just above the predicted upper elevational limit of *Cx. quinquefasciatus* (Ahumada *et al.* 2004). Regular monthly trapping at this site in 2002 and 2003 had resulted in zero

Table 2 Multiplexing information for microsatellite loci. All loci with the same multiplex number were amplified simultaneously in PCR. One primer of each pair was labelled at the 5' end with a fluorescent label (Applied Biosystems). Primer conc. indicates the final concentration of each primer in the PCR

Locus	Multiplex mix	Size range (bp)	5' Label	Primer conc. (μ M)
CQ11	1	237–269	NED	0.2
CQ26	1	209–221	6-FAM	0.2
CQ29	1	176–188	NED	0.2
CQ41	1	139–159	6-FAM	0.2
pGT12	1	144–158	HEX	0.2
qGA12	2	113–155	6-FAM	0.1
qGT17	2	139–161	NED	0.1
QTri4	2	111–123	HEX	0.4
pGT9	3	119–153	NED	0.2
qGT4	3	145–155	HEX	0.2
qGT8	3	83–119	HEX	0.2
qGT14	4	151–314	6-FAM	0.2

and eight captures, respectively. In 2004 however, a large number of individuals were captured at this location. Because we found that the 2003 and 2004 samples from this site had different microsatellite signatures, they were analysed separately.

DNA extraction

Samples from the earlier study were processed using a phenol–chloroform method (Fonseca *et al.* 2000). For mosquitoes processed specifically for this project, DNA was also extracted using a phenol–chloroform protocol, but following Anthony *et al.* (2000). Depending on the size of the resulting pellet, the DNA was resuspended in 50–200 μ L TE solution (10 mM Tris pH 7.5, 0.1 mM EDTA).

Microsatellite amplification and sizing

Each individual mosquito was typed at 12 microsatellite loci, which were originally isolated in either *Cx. quinquefasciatus* or *Cx. pipiens* (Table 2; Fonseca *et al.* 1998; Keyghobadi *et al.* 2004; Smith *et al.* 2005). By multiplexing primers we were able to amplify all 12 loci in only four polymerase chain reactions (PCR; Table 2). Each reaction occurred in a total volume of 20 μ L and contained 1 \times GeneAmp buffer (Applied Biosystems), 150 μ g/mL BSA, 2.5 mM $MgCl_2$, 200 μ M of each dNTP, 1 unit of AmpliTaq DNA polymerase (Applied Biosystems), and 1 μ L of DNA template. Up to five pairs of primers were combined in each reaction, and concentrations of individual primers varied from 0.1 μ M to 0.4 μ M, as described in Table 2. One primer of each pair was labelled at the 5' end with a fluorescent tag (6-FAM,

NED, or HEX). Thermal cycling was performed on an MJ Research Peltier machine as follows: 5 min at 96 $^{\circ}$ C; 30 cycles of 30 s at 96 $^{\circ}$ C, 30 s at 54 $^{\circ}$ C, 30 s at 72 $^{\circ}$ C; and 5 min at 72 $^{\circ}$ C. The PCR products were detected and sized on an Applied Biosystems 3100 Genetic Analyser, and alleles were called using GENOTYPER 2.5 software (Applied Biosystems).

Data analysis

Hardy–Weinberg and linkage disequilibrium. Individual loci at each sample site were tested for conformity to Hardy–Weinberg expectations using GENEPOP (Raymond & Rousset 1995). We also used GENEPOP to test for linkage disequilibrium for each pair of loci within each site. The program MICROCHECKER (van Oosterhout *et al.* 2004) was used to estimate null allele frequencies for any loci that appeared to possess null alleles, and to assess any other potential genotyping errors, such as those due to stutter or large-allele dropout.

Genetic differentiation and assignment tests. We used a number of different approaches to assess the degree of differentiation among local populations of *Cx. quinquefasciatus* on Hawaii. Pairwise tests of allele frequency differentiation between sample sites were performed using GENEPOP. Fixation indices for all of the populations, and for subgroups were calculated as in Weir & Cockerham (1984) using FSTAT (Goudet 1995).

Based on the observed alleles frequencies in each of the populations we used the assignment test (Paetkau *et al.* 1995), as executed by the program DOH (Brzustowski 2002), to assign each sampled mosquito to the population in which the individual's multilocus microsatellite genotype has the highest likelihood of occurrence. The results of the assignments were summarized for each pair of populations as the number of individuals sampled in population A and assigned to population B. To test the null hypothesis that there was no genetic differentiation among the populations, we used the program DOH to randomize the data by creating new genotypes from the combined allele pools of all of the populations, and recalculate the population assignments for each randomized data set. We could then evaluate the probability, under the null hypothesis, of an equal or greater number of assignments than we observed in our data set, from any population to another.

Isolation by distance and effects of elevation. We examined patterns of isolation by distance across all of Hawaii, and within the East region of the island where a clear elevation gradient occurs, but not within the West or North regions because the latter two contained only a small number of sample sites. We examined the correlations between genetic distance and geographical distance matrices using

Mantel tests (Mantel 1967) as executed by the program IBD (Bohonak 2002) using 10 000 matrix randomizations. For these analyses, we used Nei's standard genetic distance (Nei 1972), which performs very well for detecting isolation by distance at relatively small spatial scales and displays low variance (Paetkau *et al.* 1997). Geographic distances between sample sites were determined from their latitude and longitude co-ordinates using the GEODIS option of the R package (Legendre & Vaudor 1991).

To determine if elevation plays a role in structuring populations of *Cx. quinquefasciatus* on Hawaii, we calculated the absolute difference in elevation between each pair of sites from 1 : 100 000 US Geological Survey topographic maps with elevation contour intervals of 40 m, and constructed a matrix of pairwise elevation differences between sites. We then assessed the effects of elevation on genetic distance using Mantel tests and, where necessary, partial Mantel tests (Smouse *et al.* 1986) as executed by IBD, to control for the effect of geographical distance. In all of the above IBD analyses, only one sample from the CJ Ralph site (from 2004) was used to avoid having two samples separated by a geographical distance of zero.

We also tested for differences in measures of genetic diversity (allelic richness, observed heterozygosity, and expected heterozygosity) and *F*-statistics between low and mid-elevation sites using FSTAT. To be consistent with others working in this system, we divided the sampled populations into three different elevation classes following Ahumada *et al.* (2004) and Woodworth *et al.* (2005): Low (0–305 m), Mid (950–1350 m) and High (> 1475 m). Five populations occurred at intermediate elevations between the low and mid classes and these were excluded from the analysis because they were too few to include as a separate class. Furthermore, because there were only three samples at high elevation, and two (Keauhou and CJ Ralph 2003) had very small sample sizes, meaningful comparisons could only be made between the low and mid elevation classes.

Bottleneck analyses. Populations of *Cx. quinquefasciatus* at higher elevations experience dramatic size fluctuations (Ahumada *et al.* 2004). Furthermore, forest-dwelling mosquitoes may naturally be more susceptible to local extinctions during drought years than their domestic counterparts. Therefore, we may expect to see some signature of recent genetic bottlenecks within our high and mid elevation, and/or forest samples. Recent population bottlenecks may be detected by heterozygosity values that are higher than expected, given the number of alleles (Nei *et al.* 1975). We used the program BOTTLENECK (Cornuet & Luikart 1996) to compare, for each locus in each population, the observed heterozygosity to the expected heterozygosity obtained from simulations of the population under mutation–drift equilibrium, and given the observed

number of alleles and the sample size. Because size variation at some of these loci is caused by indels in the flanking regions and not exclusively by changes in microsatellite repeat number (Smith *et al.* 2005), we used both the infinite allele model (IAM) and the two-phase model (TPM) of mutation (with default parameters) in the simulations. For each locus, repeated simulations allow derivation of a distribution of expected heterozygosities and a statistical test of the null hypothesis that no bottleneck has occurred. A sign test or Wilcoxon sign-rank test are then used to determine if a significant majority of loci in a population exhibit greater heterozygosity than expected (Cornuet & Luikart 1996).

Origin of high elevation mosquitoes. To determine the potential source of the individuals captured above the upper predicted altitudinal limit of *Cx. quinquefasciatus*, assuming that they are all vagrants from one or more lower sites, we entered all such individuals (i.e. sampled from CJ Ralph or Keauhou) as samples of unknown origin into a separate assignment test analysis. This analysis would indicate from which other population(s) those individuals are most likely to have come.

Results

Hardy–Weinberg and linkage disequilibrium

Testing for Hardy–Weinberg equilibrium at each locus in each population, with a type I error rate of 0.01 for each test, resulted in 41 significant tests out of 432. All of the significant tests involved homozygote excess and the majority were associated with locus qGT14 (24 significant tests) or with the CJ Ralph 2004, Kealakekua and Kohala populations (four, five, and two significant tests, respectively). The observation of many cases of excess homozygosity at a single locus (qGT14) suggested the presence of null alleles at this locus (Callen *et al.* 1993). Although selection at this locus is also a possible explanation for the deviations from equilibrium, both the observation of null alleles in broader microsatellite surveys of *Culex* mosquitoes (Smith *et al.* 2005), and the generally presumed neutrality of dinucleotide microsatellites suggest that null alleles are the more likely cause. This locus has a large allele size range however, raising the possibility that the observed excess homozygosity could also be due to dropout of large alleles. Analyses by MICROCHECKER, which detect large allele dropout as an excess of homozygotes at the extreme ends of the allele size distribution, did not indicate this phenomenon. Thus, the excess observed homozygosity is most likely due to one or more mutations in the primer-binding regions that lead to nonamplifying alleles. Because the null allele frequency was quite high in some populations [estimated frequencies (Chakraborty *et al.* 1992) ranged

from 0.07 to 0.37; Table 1], we performed all analyses both with and without locus qGT14 to ensure that our results were not being biased by the presence of the null allele. Unless otherwise stated, exclusion of this locus did not alter our results. For the assignment test, which calculates the probability of occurrence of all multilocus genotypes in the different populations, locus qGT14 had to be omitted because the existence of a null allele means that genotypes at this locus are not fully known (i.e. true homozygotes and heterozygotes with a null allele are confounded).

Testing for linkage disequilibrium for each pair of loci in each population, with a type I error rate of 0.01 for each test, a number of tests were significant in the CJ Ralph 2004, Kealakekua, Kipuka Culex, and Kohala populations (6, 12, 11, and 24 significant tests, respectively). The significant tests did not consistently involve specific pairs of loci. The combined observations of excess homozygosity at two or more loci and linkage disequilibrium in the CJ Ralph 2004, Kealakekua, and Kohala samples indicate that in all three cases, individuals from separate breeding populations may have been combined in a single sample (i.e. a Wahlund effect). In contrast, our failure to reject the null hypothesis in both Hardy–Weinberg and linkage tests for low- and mid-elevation 'Biocomplexity' samples suggests that these samples were homogeneous and therefore, the pooling of individuals from separate traps within the 1-km square study grids was appropriate.

Genetic differentiation and assignment tests

We observed significant differences in allele frequency distributions between most population pairs. Even with a very conservative Bonferroni correction (setting $\alpha = 0.00008$), 266 of the 630 pairwise tests of allelic differentiation, across all loci, were significant. Genetic differentiation among all populations, as measured by F_{ST} , was significant ($F_{ST} = 0.022$; $P < 0.0001$). Within each of the East, North, and West regions, F_{ST} was also significantly greater than zero ($F_{ST} = 0.014, 0.031$, and 0.046 , respectively; $P < 0.0001$ for all).

In the multilocus assignment test, 15.5% of individuals in the data set were assigned back to their original sampled populations, meaning that the probability of occurrence of their genotype was highest in their source population. All other individuals had genotypes with a higher probability of occurrence in some other population. On the East section of Hawaii, the highest rates of self-assignment were approximately 30% in Crater Rim, Kalapana, and Rt. 137 A (Table 3). The randomization procedure of the assignment test indicated that 6 of the 36 populations had a significantly higher number of self-assignments than would be expected by chance, after a sequential Bonferroni correction for the number of tests was applied (Table 3).

Table 3 The number and proportion of individuals from each sample that were correctly assigned back to their original sample in an assignment test (Paetkau *et al.* 1995). Asterisk indicates samples that had a significantly higher number of self-assignments than would be expected by chance, if in fact all individuals formed a single panmictic population, as determined by randomization tests (after sequential Bonferroni correction). For ease of reference, each sample's location on Fig. 1 is also provided

Sample	Number assigned	Proportion assigned	No. on Fig. 1
Ainahou	1	0.100	10
Bryson's	4	0.087	1
CJ Ralph 2003	2	0.250	2
CJ Ralph 2004	8	0.174*	2
Cooper's	2	0.043	3
Crater Rim	11	0.297*	4
Hilo Airport	2	0.043	11
Hilo Golf	1	0.045	12
Hilo Zoo	3	0.150	35
Honokaa	1	0.036	13
Kalapana	2	0.286	14
Kawaihae	3	0.333	15
Keaau	2	0.044	18
Keauhou	0	0.000	16
Kealakekua	18	0.900*	17
Kipuka Culex	4	0.182	19
Kohala	20	0.556*	20
MalamaKi	5	0.111	5
Manuka	1	0.167	21
MaunaLani	0	0.000	22
McCandless1	3	0.150	23
McCandless2	7	0.206*	24
McCandless3	2	0.100	25
McCandless4	6	0.214*	26
Mt. View	0	0.000	27
Nanawale	4	0.087	6
Pahoa	2	0.200	28
Panaewa	0	0.000	29
Pepeekeo	1	0.026	30
Puu Makaala	2	0.083	31
Puu Unit	0	0.000	7
RT.137 A	2	0.286	33
RT.137B	1	0.125	32
Village	2	0.041	8
Waiakea	4	0.087	9
Waiakea Hlth Ctr.	2	0.182	34

Isolation by distance and elevation

Across all of Hawaii, there was a significant positive correlation between Nei's standard genetic distance and geographical distance ($r = 0.40$, $P < 0.0001$; Fig. 2a), but there was not a significant correlation between genetic distance and the elevation difference between pairs of sites ($r = 0.03$, $P = 0.25$).

Examining populations only on the East side of the island, where most of our sample sites were located,

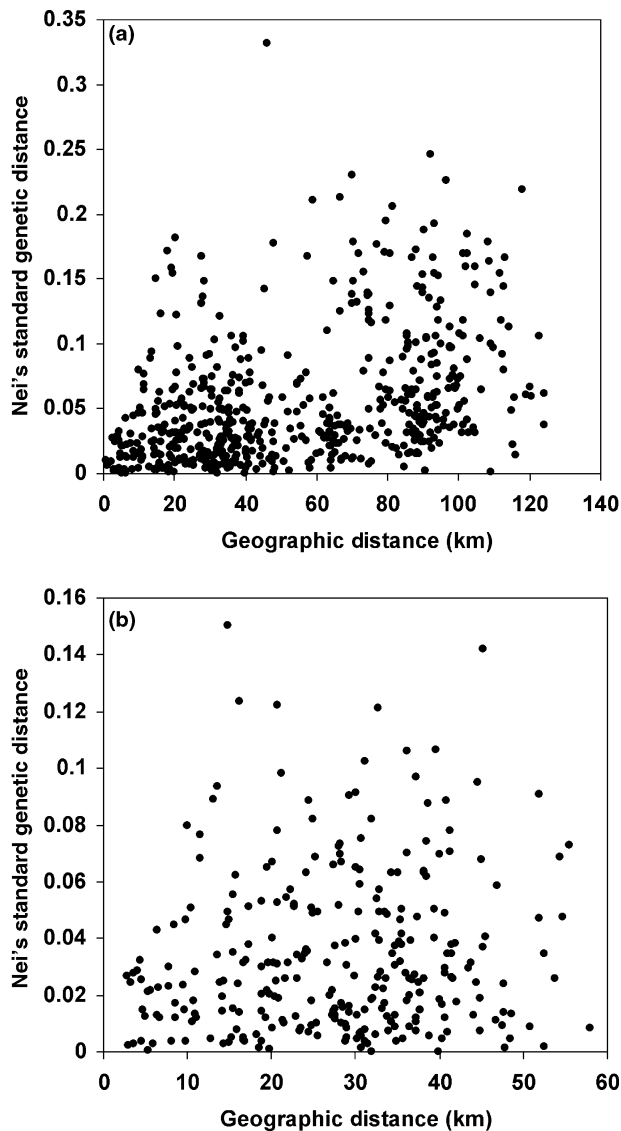


Fig. 2 Isolation by distance (Nei's standard genetic distance vs. geographical distance): (a) among all populations sampled on Hawaii, and (b) among populations sampled from the East side of the island only. Each point represents a pair of populations.

there were significant relationships between genetic and geographical distances ($r = 0.16$, $P = 0.04$; Fig. 2b), and between genetic distance and elevation difference ($r = 0.18$, $P = 0.03$). However, the effect of elevation difference on genetic distance was not significant once the shared association with geographical distance was controlled for ($r = 0.10$, $P = 0.16$).

Comparison of genetic structure at different elevations

Examining samples from all regions of Hawaii, we found that mid-elevation populations had significantly lower

Table 4 Comparisons of genetic diversity (averaged over sites) and genetic structure between low and mid elevation sites, both across the entire island of Hawaii and on the East side only. Allelic richness is calculated according to Petit *et al.* (1998). H_O and H_E are observed and expected heterozygosity, respectively. F_{ST} measures genetic differentiation. 'IBD r ' reports the correlation coefficient and P value from a Mantel test of genetic vs. geographical distances. Bolding indicates that the variable differed significantly between low and mid elevation populations. Significant differences in diversity measures and F_{ST} were determined using FSTAT (Goudet 1995). Significant differences in IBD were assessed by non-overlapping 95% confidence intervals for r^2 , estimated using bootstrapping (Bohonak 2002)

Elevation class	Allelic richness	H_O	H_E	F_{ST}	IBD r
Entire Island					
Low	3.75	0.595	0.620	0.009	0.23 ($P = 0.12$)
Mid	3.59	0.565	0.588	0.019	0.78 ($P = 0.0008$)
East Side					
Low	3.75	0.595	0.617	0.008	0.06 ($P = 0.31$)
Mid	3.61	0.561	0.588	0.006	0.03 ($P = 0.41$)

measures of genetic diversity (allelic richness, observed heterozygosity, and expected heterozygosity) than low-elevation populations (Table 4, $P = 0.017$, $P = 0.02$, and $P = 0.001$, respectively). Genetic differentiation, as measured by F_{ST} , was slightly higher among mid- than low-elevation populations; however, this difference was not significant (Table 4, $P = 0.19$). There was a significant pattern of isolation by distance among mid-elevation populations, but not among low-elevation populations (Table 4).

When only populations on the eastern side of Hawaii were considered, mid-elevation sites still had significantly lower measures of genetic diversity (allelic richness, observed heterozygosity, and expected heterozygosity) than low-elevation sites (Table 4, $P = 0.042$, $P = 0.013$, and $P = 0.003$, respectively). On the eastern side of the island, F_{ST} was almost identical between mid- and low-elevation sites (Table 4, $P = 0.80$), and there was no significant pattern of isolation by distance among populations in either elevation class (Table 4).

Bottleneck analyses

Of 29 populations with sufficient sample sizes ($n = 10$) to allow for the analysis, two populations (MaunaLani and Kealakekua) had a significant indication of a bottleneck when compared to simulations with an IAM, after a sequential Bonferroni correction for the number of tests. Also using the IAM, when locus qGT14 was excluded from the analysis, three populations had significant indications of a bottleneck after the adjustment of alpha (MaunaLani,

Pepeekeo, and Keaau). When we used the TPM of mutation, no populations had a significant bottleneck signature, after correcting for the number of tests.

Origin of high elevation (CJ Ralph) mosquitoes

When we entered the high-elevation samples (CJ Ralph and Keauhou) into an assignment test as samples of unknown origin, no other population(s) stood out as likely sources of these mosquitoes. The assignments of the individuals were evenly spread among all of the populations, with only one to three individuals assigned to any other population. The single exception was Rt. 137B, to which seven of the CJ Ralph 2004 individuals were assigned.

Discussion

Spatial structure and elevation as a barrier to gene flow

We found moderate, yet significant spatial genetic structuring among populations of *Culex quinquefasciatus* on the island of Hawaii, at smaller scales than had previously been demonstrated (Fonseca *et al.* 2000). We observed significant differences in allele frequency distributions between many population pairs, nonzero F_{ST} values, and isolation by distance, both across the entire island and within regions. Also, the assignment tests showed that a number of the samples had significantly more individual genotypes assigned to the population of origin than expected by chance, further supporting the absence of a single panmictic population. Our estimates of differentiation (F_{ST}) among populations within the East, North, and South regions of Hawaii are of similar magnitude to values obtained by Fonseca *et al.* (2000) for comparisons between regions within the island, or between different islands in the archipelago; for example, we observed a similar degree of differentiation among populations on the eastern side of Hawaii as Fonseca *et al.* (2000) observed between Maui and Oahu. *Cx. quinquefasciatus* on the island of Hawaii clearly does not form a single panmictic population, despite its purportedly rapid spread across the island since introduction (Warner 1968). However, controlling for the effect of geographical distance, we found that the elevation difference between pairs of populations did not affect their degree of genetic differentiation. Thus, elevation gradients in themselves do not appear to act as barriers to mosquito gene flow.

Because our samples were collected over a span of several years, and because some local populations of *Cx. quinquefasciatus* on Hawaii may be small and experiencing genetic drift, it is possible that the genetic differentiation we observed among samples represents confounded effects of spatial and temporal variation in allele frequencies. However, two lines of evidence suggest that the differenti-

ation we observed reflects primarily the spatial component. First, if temporal variation were contributing to observed differentiation, we would expect our estimate of differentiation to be smaller for samples collected over a shorter time. But, when we restricted the analyses to only samples that were collected within a 2-year time span [i.e. samples of Fonseca *et al.* (2000) and of HDHO, 1997–99] we actually observed slightly greater differentiation among samples ($F_{ST} = 0.028$, $P < 0.0001$). Second, some of the populations that are most likely to be subject to significant genetic drift are mid-elevation, forest-dwelling populations because they are often smaller and more subject to size fluctuations (Ahumada *et al.* 2004). For the two such populations for which we had samples collected at two different time periods (Waiakea and Crater Rim), we observed no significant differences between samples collected at different times as indicated by nonsignificant deviations from Hardy–Weinberg (see Results) and tests of allele frequency differentiation ($P = 0.46$ and 0.73 , respectively). Thus, it seems unlikely that temporal variation is outweighing spatial variation in our data set and leading to inflated estimates of population differentiation.

Differences in genetic diversity and structure between low and mid elevations

We found a significant reduction in genetic diversity in mid elevation populations compared to low elevation populations, both across the entire island and on the East side. This effect can be ascribed to the fact that, as noted above, populations become smaller and develop more cyclic dynamics with increasing elevation (Ahumada *et al.* 2004). Both factors are predicted to reduce effective population size and thereby, levels of genetic diversity.

Along with the reduction in effective population size, elevation can also affect movement rates. Being poikilothermic, the activity levels of mosquitoes depend on ambient temperatures (Rowley & Graham 1968), and thus, activity levels and movement rates are expected to decline with increasing elevation. Furthermore, larval habitat for mosquitoes becomes increasingly fragmented at higher elevations on Hawaii (Goff & van Riper 1980; van Riper *et al.* 1986), which may also lead to reduced movement. The potential for fragmented habitat structure to reduce both movement rates and gene flow has been documented in numerous studies (Templeton *et al.* 1990; Fahrig & Merriam 1994). However, we observed only a moderate effect of elevation class on differentiation among populations across the entire island, and no such effect on the East side. Thus, rates of movement and genetic exchange do not appear to be affected appreciably by temperature. Alternatively, other mitigating factors may be at play. In particular, mid-elevation populations, because of their more fragmented habitat and cyclical dynamics, may be

more subject to local extinctions and recolonizations. Such metapopulation dynamics can lead to a greater apparent gene flow among populations, if recolonizations occur from multiple source populations (Whitlock & McCauley 1990).

Bottlenecks in mid-elevation, forest-dwelling populations

Contrary to our expectations, the significant bottlenecks we detected were in larger, low-elevation populations associated with human habitation rather than in mid-elevation, forest populations. These low-elevation bottlenecks are most likely due to either population crashes caused by insecticide use or the recent introduction(s) and possible stepping-stone expansion of the mosquito that have occurred over the past 150 years. Bottlenecks as a result of size fluctuations, or extinction and recolonization events, may still occur among higher elevation or forest populations on Hawaii. However, their genetic signature may be very short-lived and therefore difficult to detect if the numbers of founding individuals are very small, as the duration of the signal is dependent upon the effective size of the founding population (Cornuet & Luikart 1996). Clearly, detailed studies of possible local extinction-colonization events in Hawaiian *Cx. quinquefasciatus* are required to determine what role they may play in structuring genetic diversity.

Origin of high-elevation mosquitoes

Population models predict that the upper altitudinal limits for *Cx. quinquefasciatus* on Hawaii are (i) 1475 m for self-sustaining populations and (ii) 1715 m for temporary, summer populations that are derived from lower altitudes (Ahumada *et al.* 2004). Empirical observations by Goff & van Riper (1980) were in concordance with these predictions, as they observed larvae and pupae of *Cx. quinquefasciatus* on Hawaii up to 1350 m throughout the year and in the summer, they found evidence of larval development up to 1650 m. The specimens that form the CJ Ralph 2004 collection were obtained just above the predicted upper limit for temporary summer populations, at approximately 1786 m. The relatively large sample collected here allows us to test the hypothesis that individuals occasionally observed at these high elevations represent temporary rather than self-sustaining populations, and also allow us to make inferences about the numbers and potential identities of the lower elevation populations from which they may be derived.

The first possibility to consider when assessing the origin of a high elevation sample is that the individuals in fact represent a new introduction of the more cold-hardy sibling species *Cx. pipiens* (Harbach *et al.* 1985). This hypothesis can readily be ruled out as the two sibling species can be differentiated on the basis of their unique microsatellite signatures (Fonseca *et al.* 2004; Smith *et al.* 2005).

The observed microsatellite allele distributions for the CJ Ralph 2004 sample (online Appendix) are uniquely diagnostic of *Cx. quinquefasciatus* (Fonseca *et al.* 2006).

The most striking and unexpected feature of the CJ Ralph 2004 sample is the high level of genetic diversity it displays. Among all of the samples, it had the third highest values for both average expected heterozygosity and allelic richness (Table 1). These results are not being driven by the large size of the sample as heterozygosity is little affected by sample size (Nei 1987) and allelic richness is expressly adjusted to account for sample size (Petit *et al.* 1998). The high genetic diversity in this sample suggests that (i) it does not represent a long-term resident population at high altitude, since low temperature leads to small population sizes (supported by the sporadic availability of specimens; Goff & van Riper 1980; van Riper *et al.* 1986; Ahumada *et al.* 2004), which in turn would result in low genetic diversity, and (ii) it has not experienced a typical bottleneck associated with a single-source founder event, a conclusion supported by the bottleneck analyses. Thus, the sample from CJ Ralph 2004 must represent a 'population' established very recently, either from a large number of founders, or from founders from more than one source population.

Results of Hardy-Weinberg and linkage disequilibrium tests indicated that the sample does indeed represent individuals from at least two separate groups. The most parsimonious explanation is that the CJ Ralph 2004 sample represents temporary summer vagrants that are derived from two or more populations at lower elevations. Although there may have been some breeding among individuals locally, it has not been sufficient to create a homogeneous group that is in Hardy-Weinberg equilibrium. Genetic analysis of the CJ Ralph 2004 sample therefore supports the existence of temporary populations at altitudes of approximately 1700 m. This is further supported by the striking difference in microsatellite allele frequencies between the samples collected at CJ Ralph in 2004 and 2003 (online Appendix), suggesting different source populations in the different years. Furthermore, because larval habitat becomes more patchy at higher altitudes (Goff & van Riper 1980; van Riper *et al.* 1986), it is perhaps not surprising that mosquitoes migrating upward in the summer from different populations at lower altitudes may become concentrated in patches of larval habitat and form temporary, mixed populations as in CJ Ralph 2004. The multisource origin of the CJ Ralph 2004 sample also suggests that multisource recolonization may occur at mid altitudes, and supports the hypothesis that the lower than expected differentiation among populations at mid altitudes may be a result of such metapopulation dynamics.

The assignment test did not identify any of the other sampled populations as likely sources of the CJ Ralph 2004 sample. This may be because the source populations have not yet been sampled, in which case further collections

focused at mid altitudes are needed to shed light on the origin of these high-elevation mosquitoes. However, given the generally low number of self-assignments in our assignment test analysis, our data set may simply lack the power to identify source populations.

Conclusion

Our results show significant differentiation among populations of *Culex quinquefasciatus* on Hawaii. This has potentially important implications for the epidemiology and evolution of the disease system on these islands, because differentiation among vector populations can affect the spatial interactions of vector, host, and parasite (Reisen *et al.* 1997; LaPointe *et al.* 2005). For example, LaPointe *et al.* (2005) found some evidence of spatial variation in the susceptibility of Hawaiian *Cx. quinquefasciatus* to the avian malaria, *Plasmodium relictum*. We also demonstrate some effects of elevation on genetic structure that are consistent with known effects of elevation on population dynamics. In particular, the reduction of genetic diversity from low- to mid-elevation populations, and the temporary nature of the high-elevation population, are both consistent with greater instability of mosquito populations with increasing altitude (Ahumada *et al.* 2004), a factor that has important implications for the spatial dynamics of avian malaria in Hawaii. Of the few studies that have explicitly examined the role of elevation in structuring insect genetic diversity, most have focused on the direct effect of elevation gradients on population differentiation (Levitan 1978; Etges 1984; Burla *et al.* 1986; Liebherr 1986; Peterson 1995). Our study shows that elevation gradients can structure populations in more complex ways, with noticeable effects on population genetic signatures. Given the strong role that elevation can play in determining insect population and community dynamics (Hodkinson 2005), such effects should be common in a wide range of insect species distributed along elevation gradients.

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Supplementary material

The supplementary material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC3069/MEC3069sm.htm>

Appendix Microsatellite data set for *Culex quinquefasciatus* on the island of Hawaii, formatted for the software Microsatellite Analyser.

References

- Ahumada JA, LaPointe D, Samuel MD (2004) Modeling the population dynamics of *Culex quinquefasciatus* (Diptera: Culicidae), along an elevational gradient in Hawaii. *Journal of Medical Entomology*, **41**, 1157–1170.
- Akey BL, Nayar JK, Forrester DJ (1981) Avian pox in Florida wild turkeys: *Culex nigripalpus* and *Wyeomyia vanduzeei* as experimental vectors. *Journal of Wildlife Diseases*, **17**, 597–599.
- Anthony TG, Trueman HE, Harbach RE, Vogler AP (2000) Polymorphic microsatellite markers identified in individual *Plasmodium falciparum* oocysts from wild-caught *Anopheles* mosquitoes. *Parasitology*, **121**, 121–126.
- Atkinson CT, Lease JK, Dusek RJ, Samuel MD (2005) Prevalence of pox-like lesions and malaria in forest bird communities on leeward Mauna Loa Volcano, Hawaii. *Condor*, **107**, 537–546.
- Atkinson CT, Woods KL, Dusek RJ, Sileo LS, Iko WM (1995) Wildlife disease and conservation in Hawaii: pathogenicity of avian malaria (*Plasmodium relictum*) in experimentally infected Iiwi (*Vestiaria coccinea*). *Parasitology*, **111**, S59–S69.
- Benning TL, D. LaPointe CT, Atkinson, PM, Vitousek (2002) Interactions of climate change with biological invasions and land use in the Hawaiian Islands: modeling the fate of endemic birds using a geographic information system. *Proceedings of the National Academy of Sciences, USA*, **99**, 14246–14249.
- Bohonak A (2002) *IBD* (Isolation by Distance): a program for analyses of isolation by distance. *Journal of Heredity*, **93**, 153–154.
- Brzustowski J (2002) *DOH Assignment Test Calculator*. <http://www2.biology.ualberta.ca/jbrzusto/Doh.php>.
- Burla H, Jungen H, Bächli G (1986) Population structure of *Drosophila subobscura*: non-random microdispersion of inversion polymorphism on a mountain slope. *Genetica*, **70**, 9–15.
- Callen DF, Thompson AD, Shen Y *et al.* (1993) Incidence and origin of 'null' alleles in the (AC)_n microsatellite markers. *American Journal of Human Genetics*, **52**, 922–927.
- Chakraborty R, De Andrade M, Daiger SP, Budowle B (1992) Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. *Annals of Human Genetics*, **56**, 45–57.
- Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.
- Dine DLV (1904) Mosquitoes in Hawaii. *Bulletin of the Hawaii Agricultural Experimental Station*, **6**, 30.
- Etges WJ (1984) Genetic structure and change in natural populations of *Drosophila robusta*: systematic inversion and inversion association frequency shifts in the Great Smoky Mountains. *Evolution*, **38**, 675–688.

- Fahrig L, Merriam G (1994) Conservation of fragmented populations. *Conservation Biology*, **8**, 50–59.
- Fonseca DM, Atkinson CT, Fleischer RC (1998) Microsatellite primers for *Culex pipiens quinquefasciatus*, the vector of avian malaria in Hawaii. *Molecular Ecology*, **7**, 1613–1621.
- Fonseca D, Keyghobadi N, Malcolm C, Mehmet C, Mogi M, Schaffner F, Fleischer RC, Wilkerson RC (2004) Emerging vectors in the *Culex pipiens* complex. *Science*, **303**, 1535–1538.
- Fonseca DM, LaPointe DA, Fleischer RC (2000) Bottlenecks and multiple introductions: population genetics of the vector of avian malaria in Hawaii. *Molecular Ecology*, **9**, 1803–1814.
- Fonseca DM, Smith JL, Wilkerson RC, Fleischer RC (2006) Pathways of expansion and multiple introductions illustrated by large genetic differentiation among worldwide populations of the southern house mosquito. *American Journal of Tropical Medicine and Hygiene*, **74**, 284–289.
- Gauthier C, Tibayrenc M (2005) Population structure of malaria parasites: the driving epidemiological forces. *Acta Tropica*, **94**, 241–250.
- Goff ML, van Riper C (1980) Distribution of mosquitoes (Diptera: Culicidae) on the east flank of Mauna Loa volcano, Hawaii. *Pacific Insects*, **22**, 178–188.
- Goudet J (1995) FSTAT version 1.2: a computer program to calculate F-statistics. *Journal of Heredity*, **86**, 485–486.
- Harbach RE, Dahl C, White GB (1985) *Culex (Culex) pipiens* Linnaeus (Diptera: Culicidae): concepts, type designations, and description. *Proceedings of the Entomological Society of Washington*, **87**, 1–24.
- Hodkinson ID (2005) Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews*, **80**, 489–513.
- Keyghobadi N, Matrone MA, Ebel GD, Kramer LD, Fonseca DM (2004) Microsatellite loci from the northern house mosquito (*Culex pipiens*), a principal vector of West Nile virus in North America. *Molecular Ecology Notes*, **4**, 20–22.
- LaPointe DA (2000) *Avian malaria in Hawaii: the distribution, ecology and vector potential of forest-dwelling mosquitoes*. PhD thesis. University of Hawaii, Honolulu, HI.
- LaPointe DA, Goff ML, Atkinson CT (2005) Comparative susceptibility of introduced forest-dwelling mosquitoes in Hawai'i to avian malaria, *Plasmodium relictum*. *Journal of Parasitology*, **91**, 843–849.
- Legendre P, Vaudor A (1991) *The R Package: Multidimensional Analysis, Spatial Analysis*. Université de Montréal, Montréal.
- Levitan M (1978) Studies of linkage in populations. IX. The effects of altitude on X-chromosome arrangement combinations in *Drosophila robusta*. *Genetics*, **89**, 751–763.
- Liebherr JK (1986) Comparison of genetic variation in two Carabid beetles (Coleoptera) of differing vagility. *Annals of the Entomological Society of America*, **79**, 424–433.
- Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.
- May RM, Anderson RM (1979) Population biology of infectious diseases: Part II. *Nature*, **280**, 455–461.
- Nasci RS, Miller BR (1996) Culicine mosquitoes and the agents they transmit. In: *The Biology of Disease Vectors* (ed. Beaty BJ, Marquardt WC), pp. 85–97. University Press of Colorado, Niwot, Colorado.
- Nei M (1972) Genetic distance between populations. *American Naturalist*, **106**, 283–292.
- Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.
- Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.
- Paetkau D, Waits LP, Clarkson PL, Craighead L, Strobeck C (1997) An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. *Genetics*, **147**, 1943–1957.
- Patz JA, Reisen WK (2001) Immunology, climate change and vector-borne diseases. *Trends in Immunology*, **22**, 171–172.
- Peterson MA (1995) Phenological isolation, gene flow, and developmental differences among low- and high-elevation populations of *Euphilotes enoptes* (Lepidoptera: Lycaenidae). *Evolution*, **49**, 446–455.
- Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conservation Biology*, **12**, 844–855.
- Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Reisen WK, Lothrop HD, Lothrop B (2003) Factors influencing the outcome of mark-release-recapture studies with *Culex tarsalis* (Diptera: Culicidae). *Journal of Medical Entomology*, **40**, 820–829.
- Reisen WK, Lothrop HD, Presser SB, Hardy JL, Gordon EW (1997) Landscape ecology of arboviruses in southeastern California: temporal and spatial patterns of enzootic activity in Imperial Valley, 1991–94. *Journal of Medical Entomology*, **34**, 179–188.
- Rowley WA, Graham CL (1968) The effect of temperature and relative humidity on the flight performance of female *Aedes aegypti*. *Journal of Insect Physiology*, **14**, 1251–1257.
- Rueda LM, Patel KJ, Axtell RC, Stinner RE (1990) Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, **27**, 892–898.
- Smith JL, Keyghobadi N, Matrone MA, Escher RL, Fonseca DM (2005) Cross-species comparison of microsatellite loci in the *Culex pipiens* complex and beyond. *Molecular Ecology Notes*, **5**, 697–700.
- Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology*, **35**, 627–632.
- Tabachnick WJ, Black WC (1996) Population genetics in vector biology. In: *The Biology of Disease Vectors* (ed. Beaty BJ, Marquardt WC), pp. 417–437. University Press of Colorado, Niwot, Colorado.
- Templeton AR, Shaw K, Routman E, Davis SK (1990) The genetic consequences of habitat fragmentation. *Annals of the Missouri Botanical Gardens*, **77**, 13–27.
- van Oosterhout C, Hutchinson WF, Willis DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.
- van Riper C, van Riper SG, Goff ML, Laird M (1986) The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecological Monographs*, **56**, 327–344.
- Warner R (1968) The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. *Condor*, **70**, 101–120.
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

- Whitlock MC, McCauley DE (1990) Some population genetic consequences of colony formation and extinction: genetic correlations within founding groups. *Evolution*, **44**, 1717–1724.
- Whittaker RH (1975) *Communities and Ecosystems*. Macmillan, New York.
- Woodworth BL, Atkinson CT, LaPointe DA *et al.* (2005) Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. *Proceedings of the National Academy of Sciences, USA*, **102**, 1531–1536.

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